

AD \_\_\_\_\_

Award Number: DAMD17-02-2-0059

TITLE: Development of a Viral Biological-Threat Bioinformatics  
Resource

PRINCIPAL INVESTIGATOR: Elliot J. Lefkowitz, Ph.D.

CONTRACTING ORGANIZATION: The University of Alabama at Birmingham  
Birmingham, Alabama 35294-0111

REPORT DATE: October 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20040105 185

**REPORT DOCUMENTATION PAGE**Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY</b> (Leave blank)		<b>2. REPORT DATE</b> October 2003	<b>3. REPORT TYPE AND DATES COVERED</b> Annual (30 Sep 2002 - 29 Sep 2003)	
<b>4. TITLE AND SUBTITLE</b> Development of a Viral Biological-Threat Bioinformatics Resource			<b>5. FUNDING NUMBERS</b> DAMD17-02-2-0059	
<b>6. AUTHOR(S)</b> Elliot J. Lefkowitz, Ph.D.				
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> The University of Alabama at Birmingham Birmingham, Alabama 35294-0111  <i>E-Mail:</i> elliotl@uab.edu			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b> Original contains color plates: All DTIC reproductions will be in black and white.				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited				<b>12b. DISTRIBUTION CODE</b>
<b>13. ABSTRACT (Maximum 200 Words)</b> In response to the potential use of viruses as biological weapons, we have established the Viral Biological-threat Bioinformatics Resource (VBBR) that collects, catalogs, annotates, and analyzes genetic information related to potential viral threats. This work expands upon available knowledge of virus replication, pathogenicity, and virus-host interactions on the basis of individual protein domains, individual genes, and whole genomes. To date, we have constructed a genome and gene sequence database that has been populated with the sequence information for viruses currently listed on the NIH and CDC priority pathogen list. We have also developed a variety of analytical and visualization tools that aid in the analysis of the genomic information coded for by these viruses. Finally, the information developed as a result of this work has been made available to the scientific community through a (currently access-controlled) web site ( <a href="http://vbbr.genome.uab.edu">http://vbbr.genome.uab.edu</a> ) that supports research efforts to develop environmental detectors, diagnostics, antiviral compounds, new vaccines, and animal models in support of biodefense research goals.				
<b>14. SUBJECT TERMS</b> Biodefense, biological terrorism, bioinformatics, viruses, sequence database				<b>15. NUMBER OF PAGES</b> 13
				<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

## Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	13
Reportable Outcomes.....	13
Conclusions.....	13
References.....	
Appendices.....	

## INTRODUCTION:

In response to the potential use of viruses as biological weapons, we have established the Viral Biological-threat Bioinformatics Resource (VBBR) that collects, catalogs, annotates, and analyzes genetic information related to potential viral threats. This work expands upon available knowledge of virus replication, pathogenicity, and virus-host interactions on the basis of individual protein domains, individual genes, and whole genomes. This information has been made available to the scientific community to support current research efforts to develop environmental detectors, diagnostics, antiviral compounds, new vaccines, and animal models.

Specifically we are:

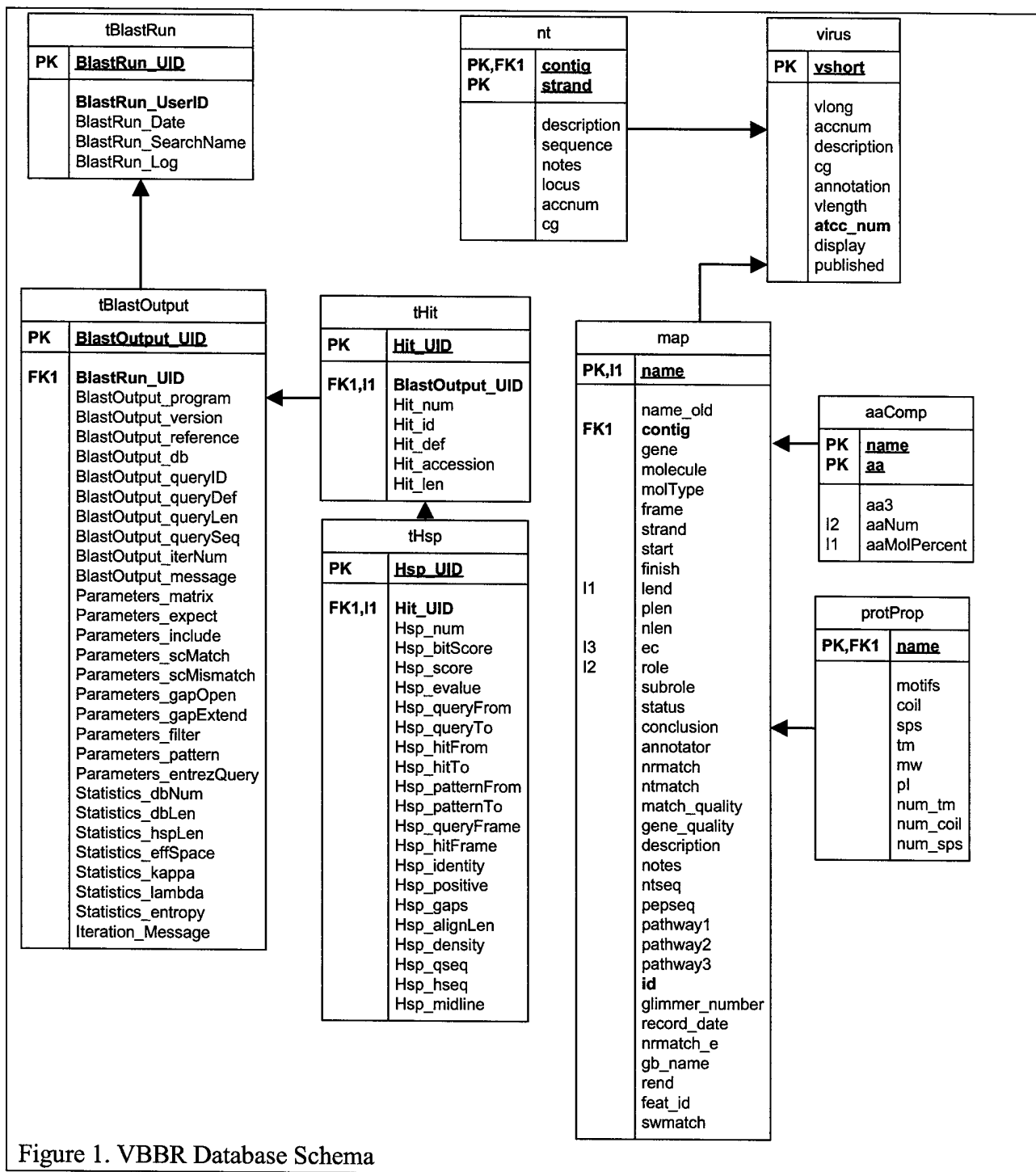
1. developing a relational database that supports the data storage, annotation, analysis, and information exchange goals of this proposal;
2. developing a world-wide web site that will allow all information and analytical results compiled and generated by this work to be publicized to the scientific community, providing rapid access to specific information as required by an individual researcher;
3. collecting existing gene and genomic sequences for viral threat agents and importing them into the database for subsequent annotation and analysis;
4. providing computer-automated and human-directed annotation to confirm and update existing biological information in the sequence records for all sequences; and
5. performing a variety of analyses on genome and gene sequences to provide additional information on their structure, function, and evolution.

## BODY:

Substantial progress has been made in accomplishing many of the tasks outlined in the original statement of work. Accomplishments extend to database construction, data population, development and implementation of analytical and visualization tools, and publication of all available information on a web site. The accomplishments are listed below, itemized according to the original statement of work and task list.

### **Task 1. To develop a relational database that will support the data storage, annotation, analysis, and information exchange goals of this proposal. (Months 1-6)**

- The database has been created based on our previous work on poxviruses and has been updated and refined to better support a wider range of virus threats. An overview of the database schema is provided in figure 1.

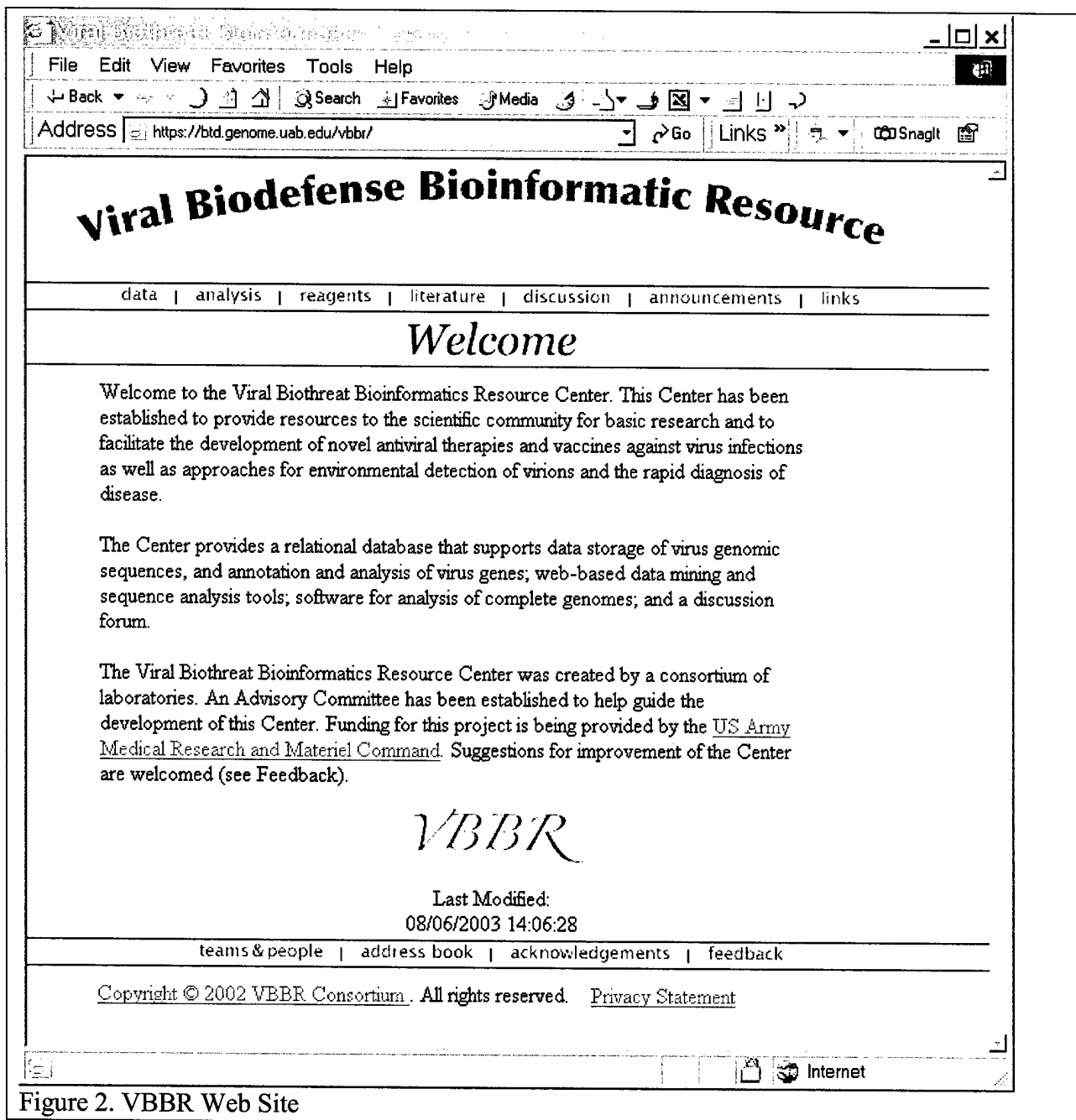


**Task 2.** To develop a world-wide web site that will allow all information and analytical results compiled and generated by this work to be publicized to the scientific community, providing rapid access to specific information as required by an individual researcher. This web site will also provide the necessary security and access controls to ensure that only individuals and groups as designated by the granting agency (USAMRMC) can utilize these resources. (Months 1-12)

- The web site has been established and is accessible at the url: <http://vbbr.genome.uab.edu>.
- Login to the web site requires registration. Following registration, a user account is established, and access is secured via a username/password. To register, a user needs to

logon to the following web page: <https://btd.genome.uab.edu/register/register.asp> using the following credentials: Username: genuab; Password: \$mgbf2003 and fill out the form. The user will be vetted for access and when approved will then be notified via Email when the account is established.

- The home page for the VBBR web site is shown below in figure 2.

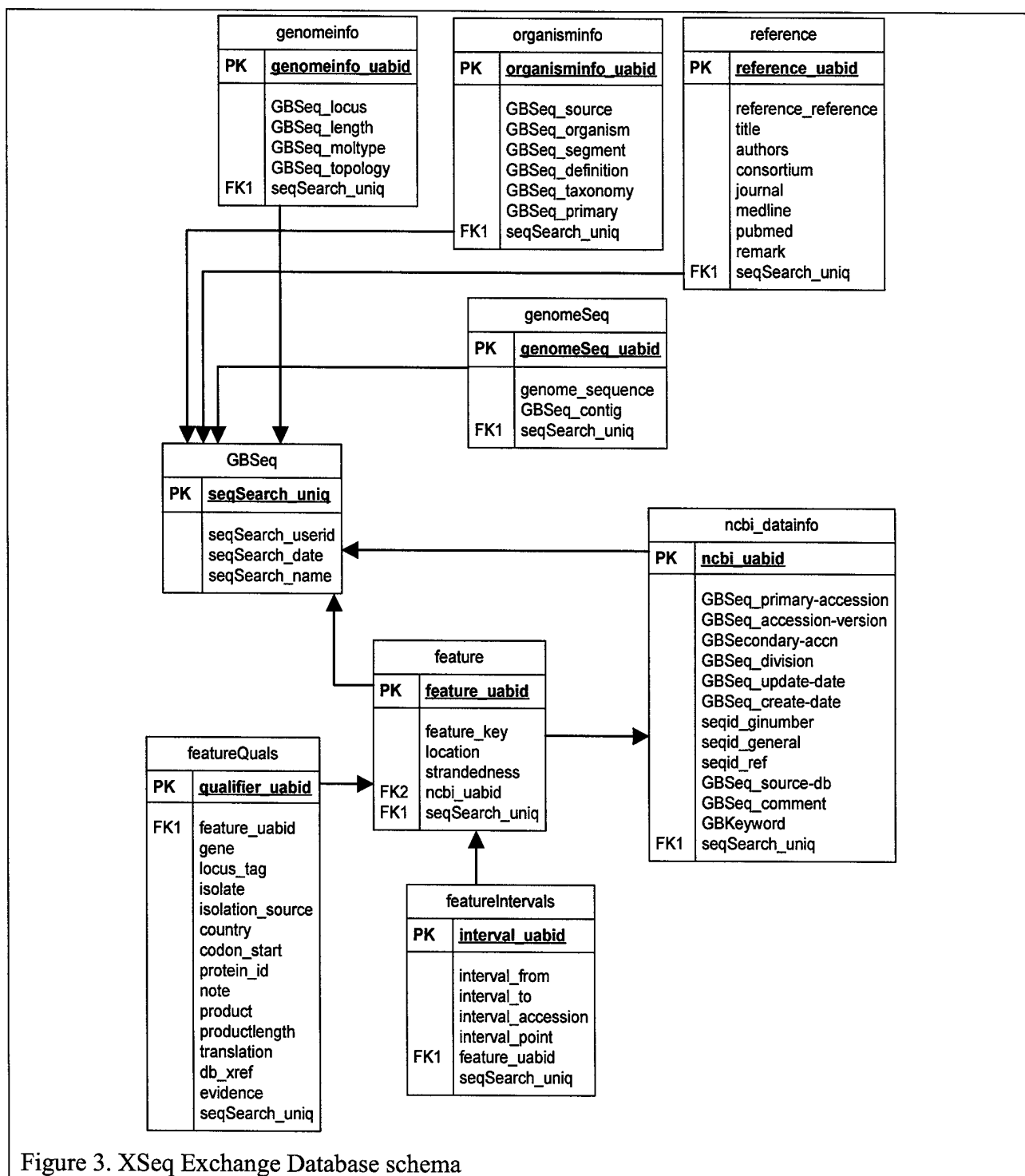


**Task 3.** To collect existing gene and genomic sequences for viral threat agents and import them into the database for subsequent annotation and analysis (Months 1-24).

- a. We will obtain existing gene and genomic sequences for all viruses that potentially could be used as agents of biological warfare or terror. The

information collected will include the corresponding descriptive annotation provided with the sequences.

- An automated parser has been developed (XSeq Exchange) that can parse XML-formatted GenBank sequence records and their annotation and load that information into the VBBR database. This application will greatly enhance our ability to add new viral genomes to the VBBR database. The database schema for the parser is shown below in figure 3.



- The genome and gene database is currently populated with most of the viruses currently on the NIAID A-C priority pathogen list. These are shown on the genomes page of the VBBR web site as displayed in figure 4.

# VBBR

[data](#) | [analysis](#) | [reagents](#) | [literature](#) | [discussion](#) | [announcements](#) | [links](#) | [home](#)

---

## Data

---

[Genomes](#) | [Genes](#) | [Sequence Download](#)

---

### Complete Genomes Available in the Viral Biodefense Bioinformatics Resource

[Click for information on changes to the VBBR virus designations.](#)

Virus (Click for Gene Map)	Complete Genome?	Genome Size (bases)	Accession Number (GenBank Accession)	ATCC Number
<a href="#">Andes virus segment L, complete sequence</a>	Y	6,562	NC_003488	
<a href="#">Andes virus segment M, complete sequence</a>	Y	3,671	NC_003467	
<a href="#">Andes virus segment S, complete sequence</a>	Y	1,871	NC_003466	
<a href="#">Crimean-Congo hemorrhagic fever virus S gene for nucleoprotein, genomic RNA</a>	N	1,672	AJ010649	
<a href="#">Crimean-Congo hemorrhagic fever virus gene for envelope glycoprotein precursor, complete cds, isolate:88166</a>	N	5,367	AB069675	
<a href="#">Eastern equine encephalitis virus, complete genome</a>	Y	11,675	NC_003899	
<a href="#">Guanarito virus strain INH-95551 segment S, complete sequence</a>	Y	3,343	AY129247	
<a href="#">Human hepatitis virus type A RNA, complete genome</a>	Y	7,474	M20273	
<a href="#">Hantaan virus S segment gene for nucleocapsid protein, complete cds, strain:Chen4</a>	Y	1,623	AB027101	
<a href="#">Hantaan virus gene for putative polymerase, genomic RNA</a>	Y	6,533	X55901	
<a href="#">Hantaan virus M segment gene for polyprotein, complete cds, strain:NC167</a>	Y	3,626	AB027115	
<a href="#">Junin virus GPC and N genes for glycoproteins and nucleocapsid protein, complete cds</a>	N	3,400	D10072	
<a href="#">La Crosse virus segment L, complete sequence</a>	N	6,960	NC_004108	
<a href="#">La Crosse virus segment M, complete sequence</a>	N	4,527	NC_004109	
<a href="#">La Crosse virus segment S, complete sequence</a>	N	984	NC_004110	
<a href="#">Lassa virus segment L, complete sequence</a>	Y	7,279	NC_004297	
<a href="#">Lassa virus segment S, complete sequence</a>	Y	3,402	NC_004296	
<a href="#">Lymphocytic choriomeningitis virus segment L, complete sequence</a>	Y	6,680	NC_004291	
<a href="#">Lymphocytic choriomeningitis virus segment S, complete sequence</a>	Y	3,376	NC_004294	
<a href="#">Machupo virus strain Carvallo segment S, complete sequence</a>	Y	3,439	AY129248	
<a href="#">Marburg virus, complete genome</a>	Y	19,112	NC_001608	
<a href="#">Tick-borne encephalitis virus, complete genome</a>	Y	11,141	NC_001672	
<a href="#">Puumala virus genomic S-RNA, isolate: Tobetsu-60Cr-93, complete sequence, viral-complementary strand</a>	Y	1,833	AB010731	
<a href="#">Rabies virus, complete genome</a>	Y	11,932	NC_001542	
<a href="#">Reston Ebola virus, complete genome</a>	Y	18,891	NC_004161	
<a href="#">Rift Valley fever virus S segment, complete sequence</a>	Y	1,690	NC_002045	
<a href="#">Rift Valley fever virus L segment, complete sequence</a>	Y	6,608	NC_002043	
<a href="#">Rift Valley fever virus, complete genome</a>	Y	3,885	NC_002044	
<a href="#">SARS coronavirus, complete genome</a>	Y	29,751	NC_004718	
<a href="#">Venezuelan equine encephalitis virus, complete genome</a>	Y	11,444	NC_001449	
<a href="#">Western equine encephalomyelitis virus, complete genome</a>	Y	11,484	NC_003908	
<a href="#">West Nile virus, complete genome</a>	Y	10,962	NC_001563	
<a href="#">Yellow fever virus, complete genome</a>	Y	10,862	NC_002031	
<a href="#">Zaire Ebola virus, complete genome</a>	Y	18,959	NC_002549	

[teams & people](#) | [address book](#) | [acknowledgements](#) | [feedback](#)

---

Copyright © 2002 VBBR Consortium. All rights reserved. [Privacy Statement](#)

Figure 4. VBBR Genomes Page.



- b. **We will also collect and provide the references for publications in the scientific literature that describe studies on the structure and function of orthopoxvirus genes and genomes. We will link this information on our web site to the appropriate gene records in our database.**
  - Not yet done. Tasked for year 2.
- c. **We will initially obtain viral sequence from public databases such as GenBank. We will also obtain unpublished sequence information through inquiries and interactions with other scientists that have been initiated by our collaborators.**
  - Not yet done. Tasked for year 2.

**Task 4. To provide computer-automated and human-directed annotation to confirm and update existing biological information in the sequence records for existing as well as newly obtained sequences. (Months 6-24)**

- Computer-automated annotation has been completed for the viruses listed in figure 4. Human directed annotation is tasked for year 2. An example annotation record is shown in figure 5.

**Task 5. To perform a variety of analyses on genome and gene sequences to provide additional information on their structure, function, and evolution. (Months 6-24)**

- Initial computer-aided analysis has been completed. Additional analyses tasked for year 2.

**Task 6. To provide through the web site, a set of analytical and visualization tools that will allow users of this resource to mine the data for useful information, to perform comparative analyses between these and other viruses, and to better visualize and assess the significance of their results. (Months 6-24)**

- Currently available analytical and visualization tools include:
  - a. Genome map visualization (Figure 6.)
  - b. Genome protein gene ortholog comparisons
  - c. Gene synteny visualization (Figure 7.)
  - d. BLAST similarity searches
  - e. BLAST search parsing and database storage
  - f. Java-based Visualization of BLAST search results (Figure 8.)
- A new database and web server has been installed and is now supporting all VBBR resources. This new server provides added security and has greatly increased performance of the VBBR web site.
- Development of additional analytical and visualization tools are tasked for year 2. These will include tools for creation and visualization of multiple sequence comparisons and tools for the inference and visualization of evolutionary trees.

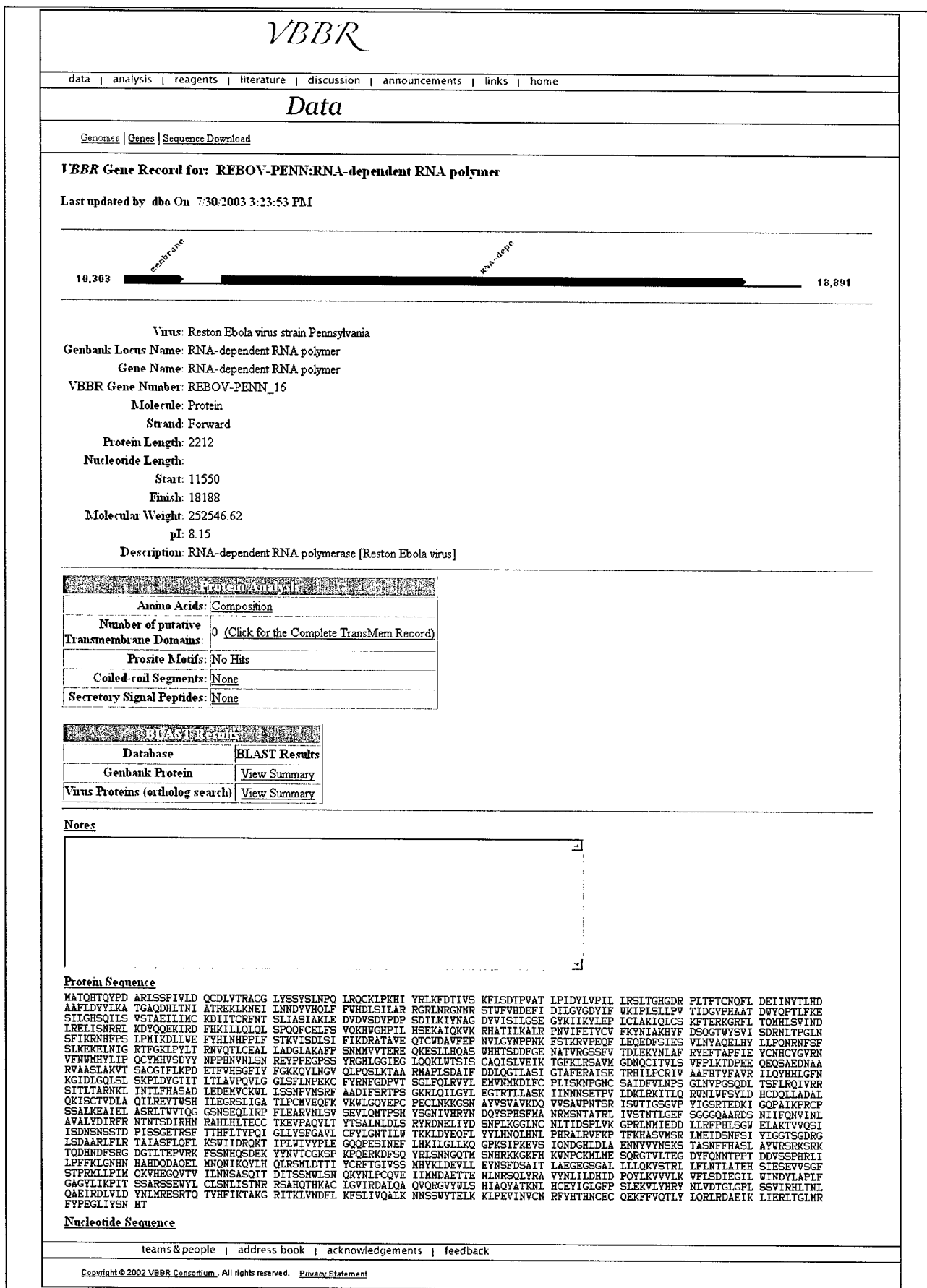


Figure 5. VBBR Gene Annotation Record.

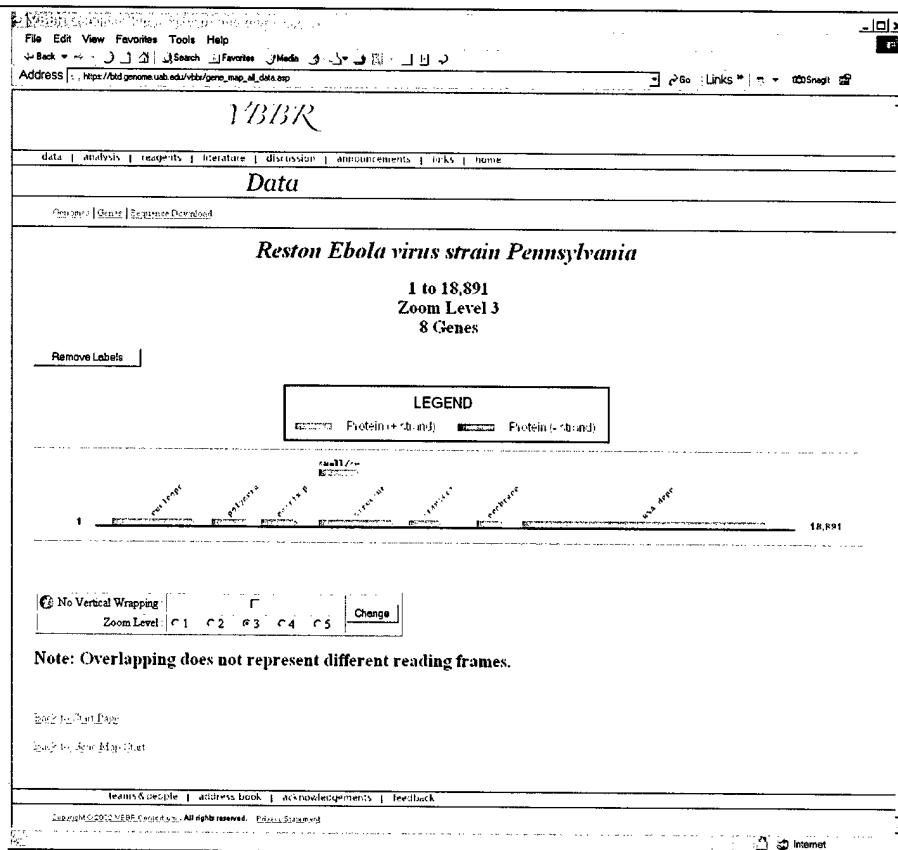


Figure 6. VBBR Genome Map.

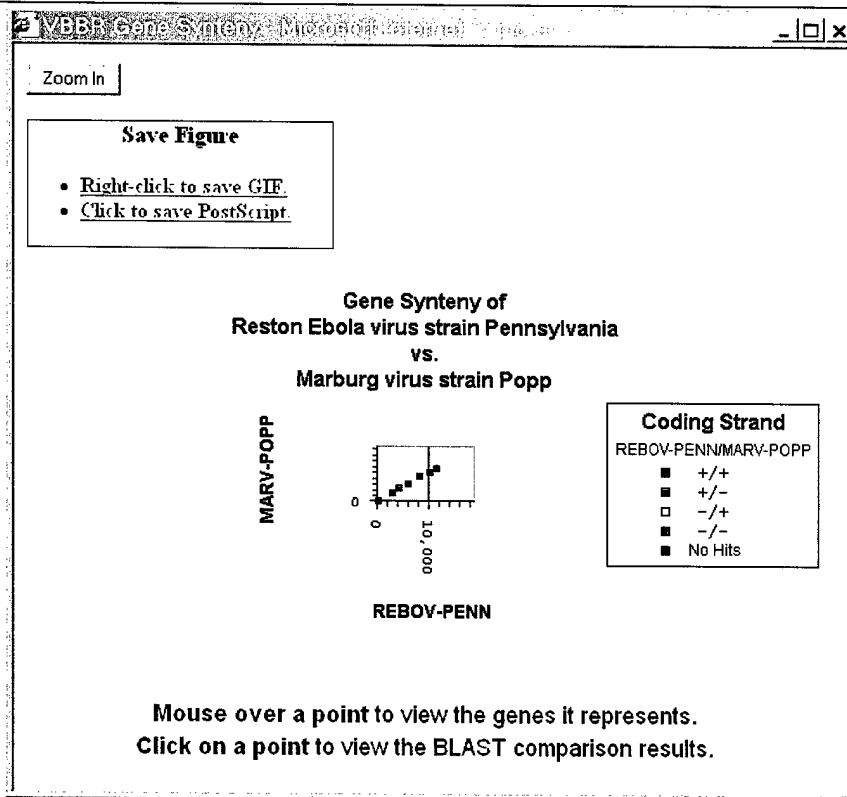


Figure 7. VBBR Gene Synteny Map.

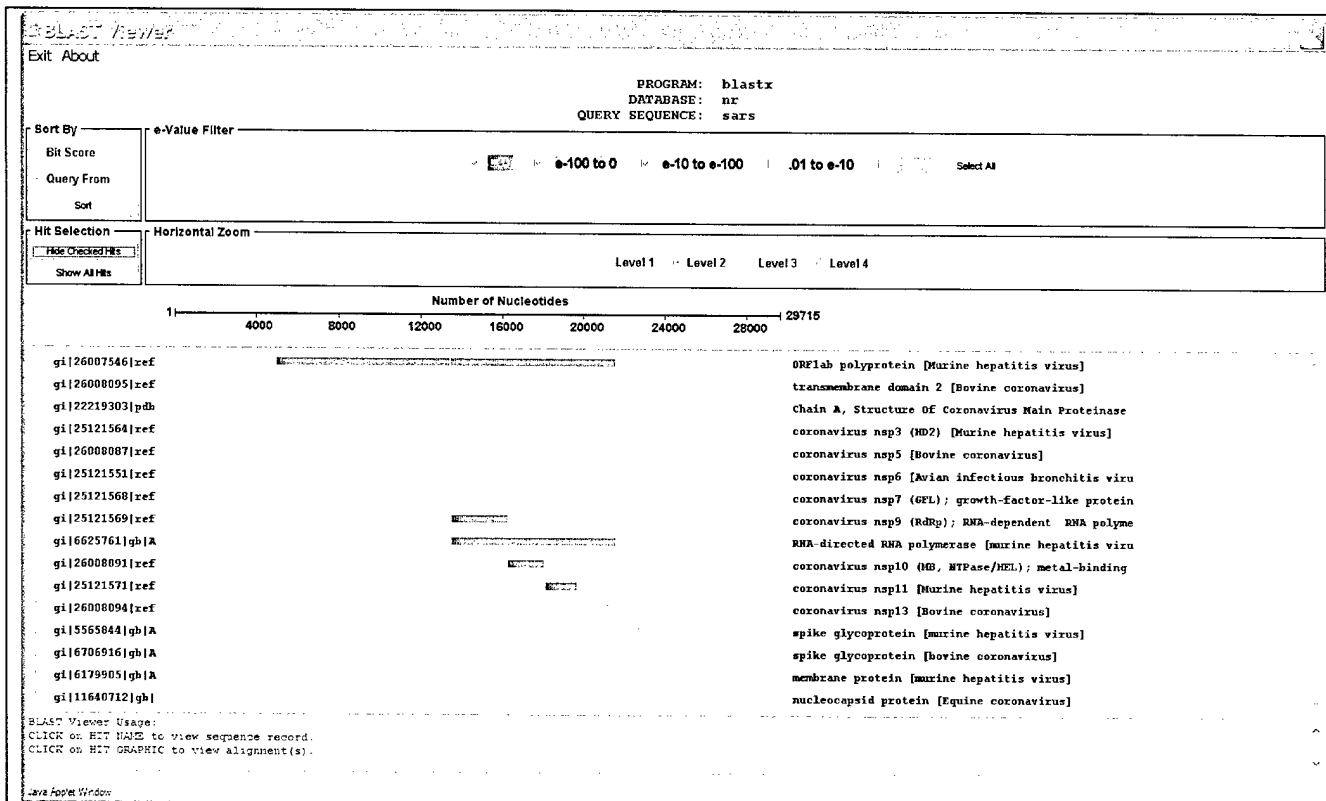


Figure 8. VBBR BLAST Viewer. The figure shows the results of a BLASTX search of the SARS genome against the Genbank non-redundant protein database.

## KEY RESEARCH ACCOMPLISHMENTS:

- Establishment of the VBBR Database
- Establishment of the VBBR Web Site
- Development of the XSeq Exchange GenBank sequence parser
- Population of the VBBR database with virus genomic information
- Computational annotation of virus genes
- Implementation of analytical and visualization tools for BLAST searches
- Implementation of analytical and visualization tools for genome ortholog comparisons

## REPORTABLE OUTCOMES:

### Informatics:

- VBBR Database
- VBBR Web site (<http://vbbr.genome.uab.edu>)
- VBBR analytical and visualization tools

### Presentations:

- USAMRIID/WRAIR/LANL/UAB Workshop, August 11, 2003 WRAIR
- USAMRMC Bioinformatics Workshop, November 4, 2003 (Poster)

### Funding proposals submitted extending this work:

- NIH/NIAID Proposal in response to the RFP: NIH-NIAID-DMID-04-34, "Bioinformatics Resource Centers for Biodefense and Emerging/Re-emerging Infectious Diseases"

## CONCLUSIONS:

Development of the Viral Biological-threat Bioinformatics Resource (VBBR) has substantially followed the original task list and essentially all tasks proposed for year 1 have been accomplished. The result is a Bioinformatics Resource Center that can now begin to support the needs of basic and applied biodefense research directed at gaining a better understanding of the pathogenesis, evolution, and overall biology of viral pathogens as well as support the development of detectors, diagnostics, antivirals, and vaccines. Future work will follow the original task list and is aimed at providing human-curated gene records along with additional analytical and visualization tools that can better support understanding the role of individual genes in virus pathogenicity.

## REFERENCES:

None other than the VBBR web site.

## APPENDICES:

None